

Effect of Prolonged Fluconazole Treatment on *Candida albicans* in Diffusion Chambers Implanted into Mice

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Fluconazole is an azole agent with primarily fungistatic activity in standard in vitro susceptibility tests. The present study was undertaken to develop a diffusion chamber model system in mice in order to study the in vivo effects of prolonged fluconazole treatment on *Candida albicans*. Chambers containing 100 *C. albicans* yeast cells were implanted subcutaneously on the flanks of C57BL/6 mice and were then retrieved 6 or 14 weeks later (after fluconazole treatment for 4 or 12 weeks, respectively). Leukocyte counts demonstrated that implantation of the chambers did elicit an inflammatory response but that only small numbers of inflammatory cells were able to enter the chamber interior. Treatment with fluconazole at 10 mg/kg of body weight/day for 12 weeks not only reduced the numbers of viable organisms within the chambers compared to those in untreated mice (mean \pm standard deviation of log₁₀ CFU of 0.7 ± 1.2 versus 2.3 ± 2.0 ; $P < 0.001$ by the Bonferroni test) but also increased the numbers of chambers that became sterile over the treatment period (14 of 16 versus 6 of 19; $P = 0.0009$ by the chi-square test). However, treatment for only 4 weeks had minimal effects on the numbers of chamber CFU, and none of the chambers became sterile during this period. Distribution of retrieved organisms between interior fluid and the chamber filters was approximately equal in all the treatment groups. This model system appears to be useful for evaluating the effects of antifungal drugs over prolonged periods in vivo. Its use in the present study demonstrates that fluconazole can increase the rate of sterilization of *C. albicans* foci that are protected from the host's inflammatory response.

Fluconazole is very useful for treating infections caused by *Candida albicans* (5, 9). At concentrations obtainable in vivo, this drug appears to have predominantly fungistatic activity in standard in vitro susceptibility tests using short incubation periods (5, 16, 20, 22, 23, 27). However, fluconazole has been found to reduce *C. albicans* viability in vitro when longer incubation periods are used (15, 22, 23). Chronic fungal infections are often long standing and may be treated for months with azole antifungal agents (6, 8, 14). Individual organisms in experimental subcutaneous *C. albicans* abscesses have been shown to proliferate relatively slowly (21), and it is likely that slow growth may be a characteristic of most chronic fungal infections. Therefore, antifungal agents used clinically may be acting on organisms that are growing slowly or not at all, with the result that individual fungal cells may be exposed to the antifungal drugs for prolonged periods.

Whereas the data from short-term susceptibility tests on antifungal agents do relate fairly well to in vivo effectiveness (1, 10, 27), certain physicochemical and pharmacokinetic factors may also play a role (27). It is not clear in the case of fluconazole whether the drug's in vivo effectiveness is related to its fungistatic or fungicidal effects or both together. In fact, in the usual experimental animal systems, it is difficult to tell if fluconazole exerts any fungicidal activity at all or if the drug acts instead by suppressing fungal growth and allowing for final elimination of the infection by the host's own defense mechanisms. Even when immunosuppressive agents are used, experimental infections rarely persist long enough to allow for ex-

tended periods of antifungal therapy as are used in human infections.

Host defenses against *Candida* are very complicated and probably involve a variety of mechanisms. Soluble mediators such as complement components and antibody may play a role, but they probably act by enhancing phagocytosis and killing by phagocytic cells (7). Since neutrophils and macrophages are critical to the defense against most fungal infections, one way to produce more chronic experimental infections would be to eliminate contact between the infecting organisms and the host's inflammatory response. Implanted chambers containing the organisms could be used for this purpose. A number of such model systems have been used to study microbial pathogenesis for various human pathogens (reviewed in reference 11). The chambers used are of two types, including tissue cages that allow entry of both humoral factors and inflammatory cells (18, 28) or diffusion chambers that permit humoral factors to enter but exclude the inflammatory cells (13, 19). In the studies described herein, we developed a Millipore diffusion chamber system that, when implanted into mice, protected the enclosed *C. albicans* cells from the host's inflammatory response and allowed the organisms to remain viable for up to 14 weeks. The purpose of the present study was to determine if fluconazole treatment for these prolonged periods could increase the rate of sterilization of the implanted chambers or if the drug's primarily fungistatic activity would suppress growth within the chambers but permit the inoculum to persist throughout the treatment period.

MATERIALS AND METHODS

Organisms. The isolate of *C. albicans* used in these studies was no. 26310 from the American Type Culture Collection (Rockville, Md.). The organisms were maintained by repeated subculture on Sabouraud's dextrose agar slants, and

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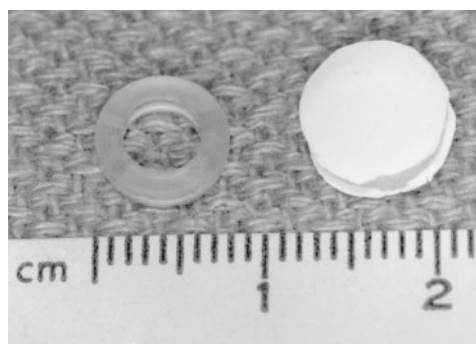


FIG. 1. Chamber ring made from 2.0-mm section of plexiglass U-100 rod (7.0-mm outside diameter) and completed chamber with 9-mm discs of 0.22- μ m-pore-size Millipore filters applied. Scale represents centimeters.

before use in the experiments, the fungal cells were scraped from 3-day-old slants and washed three times in normal saline. We have previously tested the sensitivity of this isolate to fluconazole and found the MIC of fluconazole for it to be 0.5 μ g/ml (21).

Animals. C57BL/6 mice (obtained from Charles Rivers Laboratories, Wilmington, Mass.) were used in these experiments. The animals used were males, approximately 12 weeks of age; they were housed in the Milwaukee Veterans Administration Medical Center Veterinary Medical Unit, which is fully accredited by the American Association for Accreditation and Assessment of Laboratory Animal Care.

Diffusion chambers. The chambers were fashioned as a modification of standard Millipore diffusion chambers. Plexiglas U-100 tubes, approximately 7.0 mm in outside diameter, were cut into 2.0-mm rings, and their outer edges were sanded smooth. A single hole 0.59 mm in diameter was drilled into the ring to provide access for filling of the chambers with a 27-gauge needle. Sterile 0.22- μ m-pore-size Millipore filter (MF) discs (Millipore Inc., Bedford, Mass.) cut to approximately 9 mm in diameter were cemented to each side of the ring with nontoxic MF cement from Millipore. This cement provided an impermeable and quick-drying bond that did not damage the integrity of the filters. The filter edges were left overlapping the rings to reduce sharp edges and the possibility of the chambers eroding through the skin. A chamber ring and completed diffusion chamber are shown in Fig. 1. After assembly, the chambers were ethylene oxide gas sterilized before filling and implantation. On the day of implantation, the chambers were injected with 100 *C. albicans* yeast cells in sterile saline and the hole was closed with bone wax (Ethicon, Somerville, N.J.).

Chamber implantation. Before the implantation procedure, the mice were anesthetized with sodium pentobarbital injected intraperitoneally in a dose of 50 to 80 mg/kg of body weight. The implantation sites were on the animal's flanks, slightly posterior to the scapulae, with two chambers being implanted into each animal. The skin on the back was shaved and then washed with Betadine solution, followed by sterile water. An incision of approximately 1.0 cm was made through the skin, followed by creation of a flat pocket using a sterile hemostat. After insertion of the chamber, the incision was closed with wound clips.

Chamber harvesting. After appropriate periods the animals were killed by high-dose sodium pentobarbital (100 mg/kg), and the chambers were retrieved for analysis using aseptic technique. Fluid from within the chamber was removed with a 25-gauge needle inserted through one of the end filters. Each filter was then cut out and homogenized in 1.0 ml of sterile saline in a mortar and pestle. The resulting suspensions of chamber contents or filter homogenates were appropriately diluted in distilled water and plated onto Mycosel agar for a determination of the number of CFU present. Numbers for chamber contents and filter homogenates were either pooled to give total numbers of CFU for the chamber or, in some instances, analyzed separately. A complete absence of CFU was required for a chamber to be considered sterile.

Individual experiments consisted of groups of animals (usually three or four, with six to eight chambers) that were implanted and harvested on the same day. Chambers were considered to be from different experiments if they were implanted on different days. Loss of chambers because of erosion through the skin reduced the chamber numbers, especially at the 12-week time point, with the result that some experiments represented only one or two chambers each.

Leukocyte counts. In some cases the chambers were utilized for leukocyte counts. The outside of the chamber and the pocket from which it was removed

TABLE 1. Exclusion of leukocytes by diffusion chambers^f

Chamber no. ^b	No. of leukocytes inside chambers ^a on day:					
	3 ^c	7	14	42	42F ^d	98
1	1	0	0	0	0	0
2	0	1	0	0	0	6
3	0	0	0	0	0	8
4	0	3	17	0	0	0
5	1	0	152	0	CD ^e	2
6	0	5	143	0	CD	9
7	0	1	8	0	0	0
8	0	0	0	0	0	0
9	1	0	189	0	0	309
10	0	1			0	
11	0	1			0	
12	1	0			0	
13					0	
14					0	
15					CD	
16					CD	

^a Leukocytes inside chamber, total number of leukocytes found inside the chamber.

^b Chamber number, chambers listed in order of retrieval for each time point.

^c Day, number of days after chamber implantation.

^d F, treated with 10 mg of fluconazole/kg/day for 4 weeks.

^e CD, cellular debris present but with no intact leukocytes.

^f Data come from at least four experiments per point; chambers were considered to be from different experiments if they were implanted on different days. Numbers of leukocytes (mean \pm standard deviation) found outside the chambers were $1.8 \times 10^6 \pm 1.0 \times 10^6$ ($n = 12$) on day 3, $5.2 \times 10^6 \pm 7.6 \times 10^6$ ($n = 12$) on day 7, and $3.6 \times 10^6 \pm 4.9 \times 10^6$ ($n = 20$) on day 14.

were gently swabbed with a moistened sterile cotton swab that was then agitated in a tube containing 0.5 ml of sterile saline; the leukocytes present in the saline were then counted microscopically. Interior chamber contents (about 20 μ l per chamber) were deposited onto glass slides using a cytocentrifuge (Shandon Inc., Pittsburgh, Pa.), stained using Leuko Stat (Fisher Scientific Co., Pittsburgh, Pa.), and examined microscopically. These determinations were made at 3, 7, and 14 days after implantation.

Fluconazole treatment. Fluconazole was administered to the animals in their drinking water (26). Injectable fluconazole was diluted appropriately so that 4.0 ml (the approximate amount that a 20-g mouse drinks in a day) contained the doses used of either 5.0 or 10.0 mg/kg/day. Some animals were not given fluconazole and served as controls. In preliminary experiments the quantity of water ingested by each group was measured and found to be equivalent. Fluconazole treatments were begun at 14 days after implantation of the chambers to allow for stabilization of organism growth. Treatment with fluconazole was continued until the chambers were harvested.

Statistics. Data were expressed as mean \pm standard deviation of log₁₀ CFU obtained from the chambers or as the number of chambers with any viable organisms isolated over the total number tested. Leukocytes were expressed as the number $\times 10^6$ for cells retrieved from outside the chambers or as the total number of leukocytes counted in 20 μ l of fluid from within the chambers. Mean numbers of organisms from chambers in treated versus untreated mice were compared using the two-way analysis of variance with the Bonferroni post test for significance of individual comparisons; numbers of chambers with viable organisms over the total tested were compared for treated and untreated animals using the chi-square test. Statistical significance was taken at $P < 0.05$.

RESULTS

When the interior contents were removed from inside the chambers, approximately 20 μ l of clear serous fluid was obtained at all time points tested. Washing of the chamber pocket and outside of the chambers with 1.0 ml of sterile saline yielded whitish, cloudy fluid. Individual counts of leukocytes from within the chambers are shown in Table 1. When the entire inner fluid contents of the chambers were deposited onto glass slides by cytocentrifugation and counted, very few cells (a

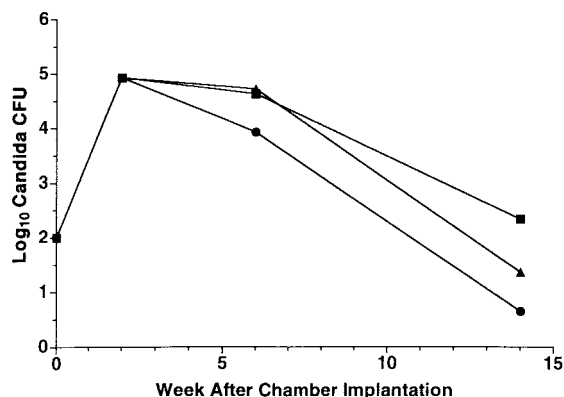


FIG. 2. Effect of fluconazole treatment on total number of CFU of *C. albicans* obtained from both the interior fluid and homogenized filters of implanted diffusion chambers during a 14-week period. Squares, control; triangles and circles, treatment with 5 or 10 mg of fluconazole/kg/day, respectively. Data come from 8 to 20 chambers (from two to seven experiments) per point; chambers were considered to be from different experiments if they were implanted on different days. The chambers contained 100 *C. albicans* yeast cells when implanted at the beginning of the experiments; treatment with fluconazole was begun at week 2 and continued until the time of chamber harvest. Treatment with 10 mg of fluconazole/kg/day produced a significant reduction in the number of CFU obtained at week 14 ($P = 0.0233$ by a two-way analysis of variance, and $P < 0.001$ by the Bonferroni correction for multiple comparisons).

range of from 0 to 309 cells per chamber) were found. Of note, there were four chambers at 42 days that showed cellular debris, without intact leukocytes. None of the other chambers showed cellular debris, although this type of material was found in a group of four chambers in which one of the filters was noted to be broken when retrieved (note that results from such visibly broken chambers were not otherwise included in the analyses). The numbers of cells washed from the outside of the chambers and from the chamber pocket were much higher (approximately 1.8×10^6 to 5.2×10^6). Differential counts of these cells from days 3 and 14 revealed them to be 91 and 94% neutrophils, respectively, with the rest being monocyte/macrophages or unidentifiable cells. Therefore, implantation of the chambers did produce a neutrophilic inflammatory response, but the chambers appeared to be fairly effective in excluding these cells from the interior.

Fluconazole treatment of the mice was begun at 2 weeks after chamber implantation and carried out for up to 14 weeks, with two doses used of 5 and 10 mg/kg/day. Figure 2 shows the data for total numbers of CFU (i.e., combined values for chamber contents and filter homogenates) obtained from the chambers at various times after implantation and fluconazole treatment. Note that numbers of CFU were highest at 2 weeks after implantation (before fluconazole treatment was begun) and then declined to the lower values obtained at 14 weeks after implantation (12 weeks of fluconazole treatment in the treated animals). At 12 weeks of fluconazole treatment, the numbers of CFU obtained from chambers in mice treated with 10 mg/kg/day were significantly reduced compared to those from control mice (\log_{10} of 0.7 ± 1.2 versus 2.3 ± 2.0 , $P < 0.001$ by the Bonferroni t test); values for the number of CFU from mice treated with 5 mg/kg/day were reduced but not significantly

TABLE 2. Sterilization of diffusion chambers containing *C. albicans* by prolonged treatment with fluconazole

Drug used (dosage)	No. of positive chambers/ no. tested (no. of expts) ^a at wk:	
	4	12
Control	11/11 (4)	13/19 (7)
Fluconazole (5 mg/kg/day)	11/11 (4)	8/20 (7)
Fluconazole (10 mg/kg/day)	10/10 (2)	2/16 (6)

^a Data represent number of chambers with viable organisms isolated over total number tested, obtained from the number of experiments in parentheses; chambers were considered to be from different experiments if they were implanted on different days. Fluconazole sterilization of the chambers at 12 weeks with the 10-mg/kg/day dose was statistically significant by the chi-square test ($P = 0.0009$).

from those in control mice. During these studies, some chambers were lost when they eroded through the skin. Up to 6 weeks after implantation, retention rates were fairly good (80% for controls and mice treated with 5 mg/kg/day and 100% for mice treated with 10 mg/kg/day); however, at 14 weeks retention rates declined significantly (values of 34, 61, and 52% for the three groups, with a mean of 46% overall).

The chamber cultures were also evaluated for the number found to be sterile compared to the total tested, as shown in Table 2. Note that, at 6 weeks after implantation (4 weeks of fluconazole treatment), all of the chambers from all three treatment groups were positive for *C. albicans* growth. However, at 14 weeks after implantation (12 weeks of fluconazole treatment), 14 of 16 chambers from the 10-mg/kg/day treatment group were sterile, versus 12 of 20 for the 5-mg/kg/day group and 6 of 19 in the control group. The numbers of negative cultures were significantly greater in the 10-mg/kg/day treatment group than in the control group according to the chi-square test ($P = 0.0009$).

The sites of the organisms obtained within the chamber (i.e., inside fluid versus homogenates of the filters) were also quantitated, as shown in Table 3. Note that, whereas the total numbers of CFU were larger in the control group, the distribution of CFU was approximately equally divided between

TABLE 3. Comparison of no. of CFU of *C. albicans* organisms obtained from chamber contents or filter homogenates in control mice and in those treated with fluconazole^c

Treatment	CFU from inside ^a	CFU from filters ^b
4 wk		
Control	4.6 ± 0.6	4.0 ± 0.9
Fluconazole, 5 mg/kg/day	4.6 ± 0.6	4.1 ± 0.4
Fluconazole, 10 mg/kg/day	4.1 ± 0.8	3.4 ± 1.2
12 wk		
Control	2.1 ± 2.0	1.9 ± 2.0
Fluconazole, 5 mg/kg/day	1.0 ± 1.5	1.1 ± 1.5
Fluconazole, 10 mg/kg/day	0.3 ± 1.1	0.4 ± 1.2

^a CFU from inside = mean \pm standard deviation of \log_{10} CFU obtained from fluid removed from chamber interior.

^b CFU from filters = mean \pm standard deviation of \log_{10} CFU obtained from homogenates of chamber filters.

^c Data are from 6 to 20 chambers (from 4 to 10 experiments) per point; chambers were considered to be from different experiments if they were implanted on different days. Note that none of the individual comparisons for numbers of CFU inside versus numbers of CFU from filters were statistically significant by the two-way analysis of variance with the Bonferroni correction for multiple comparisons.

inside fluid and filter homogenates for each of the three treatment groups. Therefore, organisms adherent to the filters do not seem to be protected from the effect of fluconazole in this model system.

DISCUSSION

In the present study we have used a diffusion chamber model system to study the effects of fluconazole on *C. albicans* in vivo during a relatively long treatment period of 12 weeks. Leukocyte counts from outside and inside the chambers demonstrated that implantation of *C. albicans*-containing diffusion chambers does elicit an inflammatory response but that most of these cells are not able to enter the chamber. Only 4 of 67 chambers analyzed for leukocytes were found to contain more than 100 such cells, in comparison to much larger numbers of viable *C. albicans* cells in the chambers (approximately 10^5 at 7 days after implantation). In four chambers, the presence of cellular debris indicated that leukocytes had entered previously but were no longer present in intact form; these were the only intact chambers in which cellular debris was found. From these results it appears unlikely that declines in numbers of viable fungal cells were due to leukocytes within the chambers. On the other hand, soluble host defense factors such as antibodies, complement, or inflammatory cell products could have entered the chambers and perhaps have affected fungal growth or viability. In previous studies we have found that simple injection of *C. albicans* yeast cells into the subcutaneous tissue on the flank of mice produces self-limited infections that generally clear promptly (1 to 3 weeks) by drainage through the skin, although small numbers of fungal cells may be left behind (24). In the present model system, the organisms persisted much longer, with most of the chambers in control animals still showing viable organisms at 14 weeks after implantation.

Treatment with fluconazole at 10 mg/kg/day for 12 weeks not only reduced the numbers of viable organisms within the chambers but also increased the numbers of chambers that became sterile over the treatment period. Distribution of retrieved organisms between interior fluid or the chamber filters was approximately equal in all the treatment groups, indicating that adherence to the filters does not protect against fluconazole's effects or prolong viability of the organisms in untreated animals. This model system appears to be useful for studying effects of antifungal drugs on viability of yeast cells over prolonged periods in vivo, although it should be noted that loss of chambers at the longer time point (14 weeks) necessitated setting up larger experiments so as to obtain sufficient numbers of intact chambers for analysis.

Animal chamber models of many types have been developed to study host-parasite interactions for a number of different organisms (11). For example, the open tissue cage models, where both humoral factors and inflammatory cells from the host are free to enter the chamber, have been used to study the movement of neutrophils into *Staphylococcus aureus* abscesses (3) and the in vivo virulence of an iron uptake mutant of *Neisseria gonorrhoeae* (12). This open type of chamber has also been employed in studies of in vivo antibiotic therapy, both in testing the efficacy of drugs against organisms in the enclosed infections and in testing the entry of antimicrobial agents into these sites (2, 4). Diffusion chambers impermeable to host cells

have also been used to study antibacterial drugs in vivo (13), and in one study of *Sporothrix schenckii* in mice, chambers were used with filters that either permitted or excluded host cells to enter the chamber (19). The major benefit of these chamber infection models over usual experimental infections appears to be ease in sampling fluids for more accurate determinations of microorganism numbers and drug levels in the infected fluids.

Our purpose in developing the *C. albicans* diffusion chamber model used in the present study was to produce infections that would persist for many weeks to allow for longer periods of fluconazole treatment. Using the chamber system, we found that fluconazole treatment at 10 mg/kg/day significantly increased the numbers of sterilized chambers after 12 weeks, whereas, after 4 weeks of treatment, none of the chambers had been sterilized in any of the treatment groups. Therefore, a long-lasting infection model did appear necessary to demonstrate this effect. It should also be noted that numbers of both organisms and chambers with viable *Candida* decreased markedly even in untreated animals. It may be that some kind of soluble host defense factors may be controlling growth of the organisms or even killing them or that fungal growth is not optimal within the chamber fluids because of a relative lack of nutrients. Another possibility is that toxic metabolites may accumulate and kill the organisms or inhibit their growth. In any event, loss of fungal cells in the control chambers is probably due to a death rate of the organisms that outstrips their proliferation.

In previous studies of subcutaneous *C. albicans* abscesses in leukopenic mice (25), we found that the pseudohyphae grew into dense focal arrays that protected the interior fungal cells from contact with the returning neutrophils when the animals recovered from their leukopenia. In these infections the masses of organisms were cleared by drainage through the overlying skin. The chamber model used in the present studies was somewhat similar to this situation in that its enclosed organisms were also protected from the host's inflammatory cells and that the chambers also could be cleared in some cases by erosion through the skin. However, since the chambers were much likelier to be retained for longer periods, they represented a better model for studying effects of prolonged antifungal treatment. The overall retention rate of the chambers in all three treatment groups at 12 weeks was 46% in this study; since each animal had two implanted chambers, the chances were good that at least one evaluable chamber would be retrieved for each animal used in this study.

Because of its longevity, this chamber model system is useful for studying the possibility that fluconazole may have some fungicidal activity in vivo if used over long enough periods. Indeed, this drug not only decreased the numbers of organisms within the chambers but also appeared to significantly increase the number of chambers that became sterilized during the evaluation period. However, it is not clear if this finding relates to the apparent fungicidal activity previously described for this drug in vitro when prolonged incubations are used. We have previously suggested that fluconazole could eliminate the organisms in chronic fungal infections by continuously preventing the fungal cells from proliferating and generating younger ones that would live longer (23). Indeed, prolonged fluconazole therapy has proven capable of clearing difficult fungal infections, such as chronic disseminated candidiasis (17); it

could do so through both its direct toxic effects on the organisms and its ability to prevent the fungal cells from generating younger, longer-lived progeny.

In summary, a diffusion chamber system in mice was developed in order to study the ability of prolonged fluconazole treatment to promote sterilization of protected *C. albicans* infections. This drug did produce an increased rate of sterilization of the chambers if therapy was continued for 12 weeks; at 4 weeks of therapy, none of the chambers had become sterile in either the control or treatment groups. The system used in the present study represents an alternative way of examining the effects of antifungal drugs on fungal cells in vivo. It is possible that certain antifungal agents with primarily fungistatic activity in vitro may reduce the viability of the organisms over long periods through direct toxic effects or by eliminating the generation of younger progeny that have longer life spans.

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REFERENCES

- Anaissie, E. J., N. C. Karyotakis, R. Hachem, M. C. Dignani, J. H. Rex, and V. Paetznick. 1994. Correlation between in vitro and in vivo activity of antifungal agents against *Candida* species. *J. Infect. Dis.* **170**:384–389.
- Arbuthnott, J. P., E. R. Arbuthnott, A. D. Arbuthnott, W. J. Pike, and A. Cockayne. 1992. Investigation of microbial growth in vivo: evaluation of a novel in vivo chamber implant system. *FEMS Microbiol. Lett.* **79**:75–79.
- Bamberger, D. M., K. M. Bettin, and D. N. Gerding. 1987. Neutrophil localization in acute and chronic experimental abscesses. *J. Lab. Clin. Med.* **109**:389–395.
- Bamberger, D. M., B. L. Herndon, and P. R. Suvarna. 1995. Azithromycin in an experimental *Staphylococcus aureus* abscess model. *J. Antimicrob. Chemother.* **35**:623–629.
- Bodey, G. P. 1992. Azole antifungal agents. *Clin. Infect. Dis.* **14**(Suppl. 1):S161–S169.
- Brass, C., J. N. Galgiani, S. C. Campbell, and D. A. Stevens. 1980. Therapy of disseminated or pulmonary coccidioidomycosis with ketoconazole. *Rev. Infect. Dis.* **2**:656–660.
- Crislip, M., and J. E. Edwards, Jr. 1989. Candidiasis. *Infect. Dis. Clin. N. Am.* **3**:103–133.
- Dismukes, W. E., R. W. Bradsher, G. C. Cloud, C. A. Kauffman, S. W. Chapman, R. B. George, D. A. Stevens, W. M. Girard, M. S. Saag, C. Bowles-Patton, et al. 1992. Itraconazole therapy for blastomycosis and histoplasmosis. *Am. J. Med.* **93**:489–497.
- Galgiani, J. N. 1990. Fluconazole, a new antifungal agent. *Ann. Intern. Med.* **113**:177–179.
- Galgiani, J. N. 1990. Susceptibility of *Candida albicans* and other yeasts to fluconazole: relation between in vitro and in vivo studies. *Rev. Infect. Dis.* **12**(Suppl. 3):S272–S275.
- Genco, C. A., and R. J. Arko. 1994. Animal chamber models for study of host-parasite interactions. *Methods Enzymol.* **235**:120–140.
- Genco, C. A., C. Y. Chen, R. J. Arko, D. R. Kapczynski, and S. A. Morse. 1991. Isolation and characterization of a mutant of *Neisseria gonorrhoeae* that is defective in the uptake of iron from transferrin and haemoglobin and is avirulent in mouse subcutaneous chambers. *J. Gen. Microbiol.* **137**:1313–1321.
- Georgopoulos, A., A. Buxbaum, and W. Graninger. 1999. Efficacy of beta-lactams and inhibitor combinations in a diffusion chamber model in rabbits. *J. Antimicrob. Chemother.* **43**:497–501.
- Graybill, J. R., D. Lundberg, W. Donovan, H. B. Levine, M. D. Rodriguez, and D. J. Drutz. 1980. Treatment of coccidioidomycosis with ketoconazole: clinical and laboratory studies of 18 patients. *Rev. Infect. Dis.* **2**:661–673.
- Gujral, S., E. Brummer, and D. A. Stevens. 1996. Role of extended culture time on synergy of fluconazole and human monocyte-derived macrophages in clearing *Candida albicans*. *J. Infect. Dis.* **174**:888–890.
- Hughes, C. E., and W. H. Beggs. 1987. Action of fluconazole (UK-49,858) in relation to other systemic antifungal azoles. *J. Antimicrob. Chemother.* **19**:171–174.
- Kauffman, C., S. Bradley, and S. Ross. 1991. Hepatosplenic candidiasis: successful treatment with fluconazole. *Am. J. Med.* **91**:137–141.
- Schaad, H. J., C. Chuard, P. Vandaux, P. Rohner, F. A. Waldvogel, and D. P. Lew. 1994. Comparative efficacies of imipenem, oxacillin, and vancomycin for therapy of chronic foreign body infection due to methicillin-susceptible and -resistant *Staphylococcus aureus*. *J. Antimicrob. Chemother.* **33**:1191–1200.
- Schaude, M., and J. G. Meingassner. 1986. A diffusion chamber technique for testing of antifungal drugs against *Sporothrix schenckii* in vivo. *J. Med. Vet. Mycol.* **24**:297–304.
- Sheehan, D. J., A. Espinel-Ingroff, L. S. Moore, and C. D. Webb. 1993. Antifungal susceptibility testing of yeasts: a brief overview. *Clin. Infect. Dis.* **17**(Suppl. 2):S494–S500.
- Sohnle, P. G., and B. L. Hahn. 1993. Inhibition of pseudohyphal growth as a neutrophil-mediated host defense mechanism against experimental deep *Candida albicans* infections in mice. *J. Lab. Clin. Med.* **121**:235–243.
- Sohnle, P. G., B. L. Hahn, and M. D. Erdmann. 1996. Effect of fluconazole on viability of *Candida albicans* over extended periods of time. *Antimicrob. Agents Chemother.* **40**:2622–2625.
- Sohnle, P. G., B. L. Hahn, T. A. Fassel, and V. M. Kushnaryov. 1998. Analysis of fluconazole effect on *Candida albicans* viability during extended incubations. *Med. Mycol.* **36**:29–36.
- Sohnle, P. G., L. Radke, B. L. Hahn, and D. K. Wagner. 1992. Inefficiency of in vivo candidacidal mechanisms in experimental subcutaneous infections with *Candida albicans*. *Infect. Immun.* **60**:3940–3942.
- Sohnle, P. G., B. L. Hahn, and D. K. Wagner. 1994. Arrays of *Candida albicans* pseudohyphae that protect the organisms from neutrophil fungicidal mechanisms in experimental infections in mice. *J. Med. Vet. Mycol.* **32**:21–30.
- Sugar, A. M., C. A. Hitchcock, P. F. Troke, and M. Picard. 1995. Combination therapy of murine invasive candidiasis with fluconazole and amphotericin B. *Antimicrob. Agents Chemother.* **39**:598–601.
- Troke, P. F., R. J. Andrews, G. W. Pye, and K. Richardson. 1990. Fluconazole and other azoles: translation of in vitro activity to in vivo and clinical efficacy. *Rev. Infect. Dis.* **12**(Suppl. 3):S276–S280.
- Widmer, A. F., R. Frei, Z. Rajacic, and W. Zimmerli. 1990. Correlation between in vivo and in vitro efficacy of antimicrobial agents against foreign body infections. *J. Infect. Dis.* **162**:96–102.